E.Z.N.A.® Plasmid DNA Mini Kit I

Revision No: 2.2

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D6942-00 D6943-00	D6942-01 D6943-01	D6942-02 D6943-02	D6942-03 D6943-03
Purifications	5	50	200	600
HiBind® DNA Mini Columns	5	50	200	600
2 mL Collection Tubes	5	50	200	600
Solution I	3 mL	20 mL	60 mL	200 mL
Solution II	3 mL	20 mL	60 mL	200 mL
Solution III	3 mL	20 mL	80 mL	250 mL
HBC Buffer	5 mL	25 mL	80 mL	250 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL	200 mL
Elution Buffer	2 mL	15 mL	30 mL	100 mL
RNase A	Pre-Added	100 µL	400 µL	1.5 mL

Supplied by user:

- Tabletop microcentrifuge capable of 13,000*g*
- Vortexer
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- 100% ethanol
- 100% isopropanol
- Optional: sterile deionized water
- Optional: water bath, heat block, or incubator capable of 70°C
- Optional: 3M NaOH solution

Before starting:

- Prepare Solution I, HBC Buffer, and DNA Wash Buffer according to the directions on the bottles.
- Set water bath, heat block, or incubator to 70°C.
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb.
- Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.

Plasmid DNA Extraction and Purification from 1-5 mL *E. coli* culture

LYSE

1. Grow 1-5 mL culture overnight in a 10-20 mL culture tube.
2. Centrifuge at 10,000*g* for 1 minute at room temperature. Decant or aspirate and discard the culture media.
3. Add 250 µL Solution I mixed with RNase A (see the bottle for instructions). Vortex to mix thoroughly. Transfer suspension into a new 1.5 mL microcentrifuge tube.
4. Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary. Avoid vigorous mixing and do not exceed a 5 minute incubation.
5. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms. Centrifuge at maximum speed (≥13,000*g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

BIND

6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

7. Transfer the cleared supernatant from Step 5 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

WASH

8. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

9. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.

Optional: Repeat Step 9 for a second DNA Wash Buffer step.

10. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

11. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

12. Add 30-100 µL Elution Buffer or sterile deionized water. Let sit at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute.

13. Store eluted DNA at -20°C.